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Short Note

# Evaluation of limiting factors of Oral Rabies Vaccinations (ORV) in wild canids, evidence from a field trial in Central Italy

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## Abstract

Over the last few decades, Oral Rabies Vaccinations (ORV) has eliminated sylvatic rabies from most of Central and Western Europe. Despite this success, they will probably be needed in the near future, due to the expansion of native and invasive reservoirs. It is unclear if they still work, at this time when many countries have an abundant wild boar population and complex assemblages of mammals. The ORV boasts a non-invasive and socially acceptable approach, but it presents some critical issues that we have analyzed in our study. Between November 2011 and March 2015, a total of 249 Rabigen® SAG2 VIRBAC baits were positioned on the ground at 5 sites in the study area. Baits were monitored by weekly inspections of camera traps. Overall, 63.4% of baits were found to have been worn after one week, mostly due to rainy weather, and had to be replaced. Remaining baits lasted on the field $3.66 \pm 1.07$ days and 54.4% of them were consumed by wild boar (*Sus scrofa*). Our data highlight how a potential reservoir species such as the grey wolf did not ingest the vaccine baits while only few were taken by red fox, due to numerous competitors, especially wild boar. Findings raise doubts about the effectiveness of ORV in areas of Europe characterized by the presence of non-target species, particularly wild boar. The rising number in wild boar populations around Europe could seriously reduce baits presence in the environment, limiting their effectiveness. Future ORV campaigns against re-emerging rabies in Europe should either account for bait loss, or experiment with targeted forms of bait distribution, that prevent their uptake by non-target species.

Rabies is an acute viral encephalitis with a mortality rate of almost 100% in humans, and also severe impacts on some rare species of carnivores (Canning et al., 2019; Sillero-Zubiri et al., 2016). The etiological agent of the disease is an enveloped single-stranded negative-sense RNA virus belonging to the family *Rhabdoviridae* and the genus *Lyssavirus* (Order *Mononegavirales*), which is transmitted through the bite of rabid animals. Although Lyssaviruses can infect many species of mammals, carnivores are the prime cause for the spread of sylvatic rabies in developed countries (Fooks et al., 2014). Over the last few decades, the incidents of wildlife rabies in Europe were greatly reduced. This is thanks to prolonged control efforts by member states (European Commission, 2017). Nevertheless, the disease remains endemic in Eastern Europe, which points out the need to improve trans-boundary surveillance systems and joint programs for its elimination (Zecchin et al., 2019). Unfortunately Western, Central and Southern Europe still host various reservoir species posing different challenges for rabies control. Among carnivores, the red fox (*Vulpes vulpes*) is widespread across natural and anthropized environments, and invasive alien species such as the raccoon dog (*Nyctereutes procyonoides*), the raccoon (*Procyon lotor*) and the American mink (*Neovison vison*) are expanding their distribution (Salgado, 2018; Kauhala and Kowalczyk, 2011; Bonesi and Palazon, 2007). Moreover, Europe is also being re-colonized by larger reservoir species, such as the grey wolf (*Canis lupus*, Chapron et al., 2014) and the golden jackal (*Canis aureus*, Lapini et al., 2021). Such a widespread increase in reservoir species can trigger the re-emergence of rabies (Thompson et al., 2009) and it calls for extensive, and effective, measures for rabies control. Traditionally, oral rabies vaccination (ORV) campaigns have been the most common and most effective measure to stamp out the disease in some European countries and in much of North America (Müller et al., 2015; Mähl et al., 2014; Slate et al., 2009). Attenuated rabies viruses, such as SAG2, lead to protective immunity as they replicate in local tissues of the oral cavity and can be eliminated rapidly (Orciari et al., 2001). Baits with SAG2 are effective at vaccinating red foxes and raccoon dogs, species which are often involved in rabies infection (Mähl et al., 2014) but it is also working with over 30 target and non-target species, including carnivores, primates, rodents, and birds (Cliquet et al., 2007). Furthermore, no vaccine-induced rabies cases were diagnosed after the distribution of over 20 million SAG2 baits in Europe (Mähl et al., 2014). Based on what we saw, extensive oral vaccinations could immunize smaller reservoirs, as well as being a valid alternative to parental vaccination in those more elusive species, such as grey wolf (Sillero-Zubiri et al., 2016). However, nowadays it is unclear if ORV in Europe would still have the same high effectiveness than that in the past (European Commission, 2017). For example, it might be argued that effectiveness could have decreased due to increased bait removal by non-target species caused by the strong rise in the populations of mammals such as wild boar (*Sus scrofa*, Massei et al., 2015) or invasive alien species, in particularly the raccoon (Salgado, 2018). Baits removal by wild boar is potentially very concerning, due to the very high densities of the species in Europe and its occurrence in urbanized environments. So far, other studies showed these concerns and confirmed that boars can consume baits (Dascalu et al., 2019; Gajda et al., 2018). However, none of them quantified the magnitude of this phenomenon. In this study, we evaluated the uptake of ORV baits by target and non-target species

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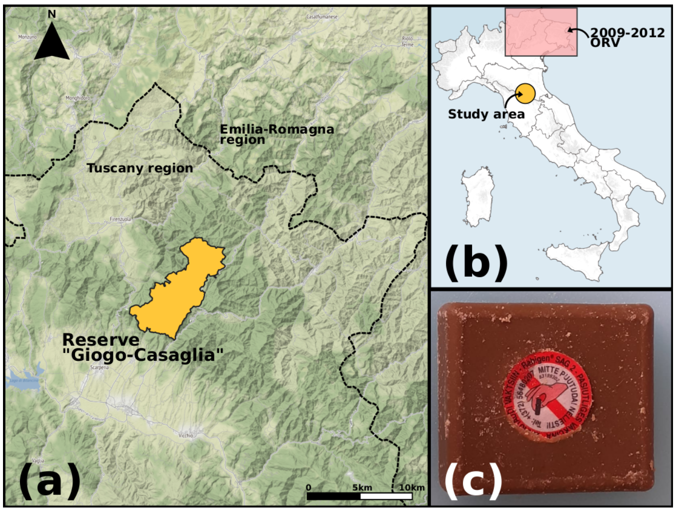


**Figure 1** – a) Map of the study area showing the position of the Reserve "Giogo-Casaglia" close to the bound-ary between Tuscany and Emilia-Romagna regions, b) Position of the study area and of the areas subjected to ORV between 2009 and 2012, in Italy and c) Example of Rabigen® SAG2 VIRBAC baits used in the study.

in Central Italy. Although the rabies virus re-emerged in neighbouring North-Eastern regions in 2008, where several ORV campaigns for red foxes were conducted in 2009–2012 (Mulatti et al., 2013, 2012; Capello et al., 2010), the area of our study is currently not affected by sylvatic rabies. Our findings raise concern about the potential removal of ORV baits by non-target species, especially eaten by the wild boar and call for i) further quantifications at larger spatial scales, ii) revaluation of the immunization of target species and iii) the development of adequate solutions for delivering ORV baits to reservoir species.

From 2011 to 2015, between November and March of each year, a total of 249 baits were placed every 7 days on the ground at 5 sites in the Reserve "Giogo-Casaglia" in Central Italy (Fig. 1). The Reserve (6100 ha) is classified as having a temperate oceanic climate, according to the Koppen climate classification. Average monthly temperatures range between 2.5 °C (January) and 21.6 °C (July), while monthly total rainfalls range between 41 mm (August) and 113 mm (November).

Data collection aimed to test bait uptakes by the grey wolf and sites were chosen in areas that had been found to be systematically frequented by the species (Apollonio et al., 2018). Baits were the Rabigen® SAG2 VIRBAC (Carros, France) in the form of oral suspension. It contains live attenuated rabies virus, minimum SAG2 strain 8 log10 DICC50*/dose (marketing authorization numbers EU/2/00/021/001 and EU/2/00/021/002). The baits used for the study were provided by a local health company in the FVG region and were a remnant of the ORV to counter the 2009 outbreak that involved eastern Italy (Mulatti et al., 2013). The capsule containing the vaccine solution was removed from all the baits used. The baits were composed of Rhodor Antifoam 7046R, HD tetracycline hydrochloride, EVA (Ethyl Vinyl Acetate), white soft paraffin, paraffin 50/52°C, Seah Saur, natural fish aroma. Baits are resistant in water and not degraded by rainfall (Mähl et al., 2014). To extract the vaccine, each bait was broken into several parts. Then, whilst the vaccines were disposed according to the special waste regulations, the bait parts were positioned in the field for the study. The distribution of these food baits took place in the cold months (from November to March), with the intention of simulating what would be a distribution of the actual vaccine baits, which require cold temperatures for their conservation. All the baits were placed on the ground at distance of 30 cm from the camera traps. Each session involved checking all the baits monitored through camera trapping, to record uptake by wildlife species. Each site was regularly checked every 6.85±2.00 day (mean ± standard deviation) to evaluate bait degradation due to climatic conditions and to replace them if necessary. For each bait we recorded whether and when it was ingested by wildlife, as well as wildlife presence and behaviour towards the bait. A single bait often was eaten by multiple species, and we considered all species eating baits pieces as involved in the consumption. The number of days required by a bait to be totally consumed was calculated as a mixed-effect Generalized Linear Model (Zuur et al., 2009), with the time of the year when a bait was positioned as a covariate. The time of the year was expressed in terms of the first or second half of each month, from November to March. This covariate captured within-season variation in the duration of baits, caused by differences in the phenology of target and non-target species. For example, species such as wild boar could reduce, or increase, their home range during the hunting season, between October and December (Scillitani et al., 2010). We also included the year and sites where baits were positioned as two non-nested random-intercept terms, to account for temporal and site-specific differences. Model selection was based on leave-one-out cross validation (Vehtari et al., 2017). Statistical analyses were carried out with STAN (Carpenter et al., 2017), and implemented on the statistical software R (R Core Team, 2021).

Firstly, we found a high degree of bait degradation: 158 (63.4%) out of 249 baits were found to be worn after approximately one week due to weather conditions, and they needed to be replaced as they had lost their characteristic scent and shape (Maciulskis et al., 2008). The high rate of degradation of the baits was worsened by rain but above all by the fact that the baits were broken for the removal of the vaccine, this practice affected the permeability and duration of the baits. Camera traps recorded 15 species frequenting baiting sites: *Dama dama* (n. interactions=478), *Sus scrofa* (n=403), *Martes* sp. (n=108), *Vulpes vulpes* (n=106), *Canis lupus italicus* (n=84), *Meles meles* (n=62), *Equus ferus caballus* (n=34), *Lepus europaeus* (n=31), *Mus musculus* (n=26), *Capreolus capreolus* (n=13), *Canis lupus familiaris* (n=12), *Hystrix cristata* (n=10), *Felix sylvestris* (n=6), *Felis catus* (n=3), *Mustela putorius* (n=1). Only 6 species fed on baits: *Sus scrofa* (n. occasions=136), *Martes* sp. (n=82), *Canis lupus familiaris* (n=12), *Vulpes vulpes* (n=10), *Hystrix cristata* (n=7), *Mus musculus* (n=3) (Fig. 2). The best candidate model had a zero-inflated Poisson distribution and the response variable had the best outcome (the reproducible dataset and software code area available at: https://osf.io/ah8u9/). This model estimated that baits disappeared after 3.66±1.07 days.

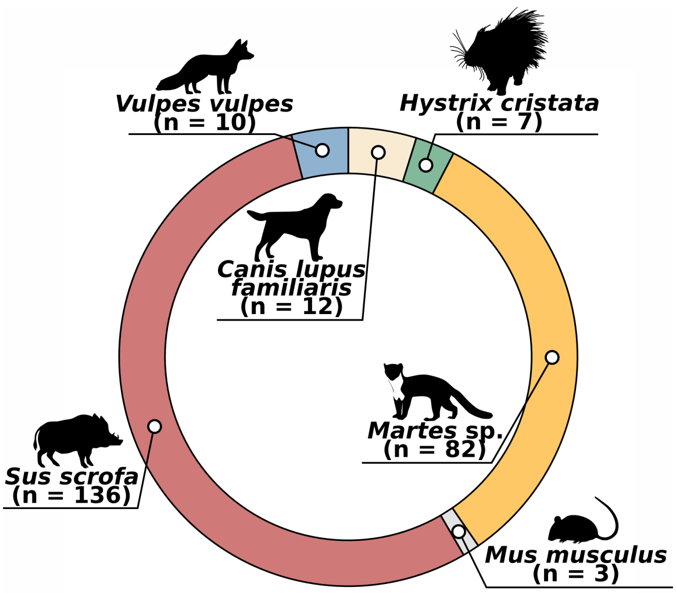


**Figure 2** – Number of occasions when baits were consumed by different wildlife species.

Our findings indicate two points, which are critical for ORV campaigns in Italy and, more broadly, in those areas of Europe characterized by the presence of wild boar and other non-target species. Firstly, among all the species that visited baiting sites, the wild boar took baits with the highest frequency (54% of feeding visits). While this matches with evidence from Eastern Europe and the Far East (Kim et al., 2020; Dascalu et al., 2019), where studies found the presence of antibodies against rabies in culled wild boar, we did not expect to record this frequency in our study. Considering that the wild boar is constantly increasing its distribution and populations in Europe (Massei et al., 2015) our findings confirms the growing concerns about the subtraction of bait by non-target species, to the detriment of reservoir species. While two or three decades ago, it was possible to eliminate rabies from Western European countries through the release of ORV baits, today this approach would probably be jeopardized by wild boar bait uptake. This problem requires the adoption of new devices, such as selective feeders (Balseiro et al., 2019) that should focus to target species only, excluding boars. While these devices are unlikely to exclude smaller non-target species, excluding the boars would significantly limit bait uptake by non-target species. Moreover, exclusion fences have already been adopted to reduce wild boar access to crops (Laguna et al., 2022), and focusing bait administration to fenced croplands is likely to further reduce their uptake by wild boar. This data, if read in reverse, could also suggest a way of vaccinating wild boar against its most impacting infectious diseases. To the best of our knowledge, no study proposed any particular solution to this problem, which should be solved as soon as possible. Especially because over the next few years, bait uptake in Central Italy is likely to be done also by invasive alien species, such as raccoons, which are now rapidly increasing their presence (Boscherini et al., 2020). Of particular interest is the expansion of the golden jackal (Lapini et al., 2021), which is not an invasive alien species but has never been reported in historical times in Italy. The species has recently expanded its range in northern Italy, with sightings also in the central Apennines. Albeit raccoons and golden jackals are a primary reservoir species, they can become proficient at removing baits — which help to reduce the spreading of rabies in those species — but at the same time they can become potential competitors, decreasing vaccine coverage of reservoirs present with greater density on the territory, as in the case of the red fox and the gray wolf. The second point was the lack of bait uptake by the grey wolf in our study area. While we recorded 84 occasions on which wolves approached baits, they never ate them. Wolves in Italy are subject to high levels of persecution (Musto et al., 2021) and therefore some of them probably show high levels of neophobia, being suspicious towards food of unknown origins, which is also contaminated with human scent. The fact that baits were rapidly degraded by environmental conditions, or consumed by boars and no-target species, probably maintained human presence, and smell, high around baiting sites and it could have contributed to deter wolves from eating baits. Future studies aim to show if bait uptake by wolves is influenced by human presence around baiting sites, and if it is different between young and adult individuals, like in the case of other forms of bait administration to canids (Kreplins et al., 2018). A noteworthy fact is that only 4% of the baits had been consumed by the red fox, which was the most important reservoir of sylvatic rabies in Italy. The red fox has a stable population on the Italian territory and nowadays the grey wolves are common in Central and Northern Italy (Bassi et al., 2015) and they could become a reservoir in case of rabies re-emergence. Understanding which factors can influence bait uptake by wild canids would be fundamental. At the same time, their trapping and vaccination, though possible, is poorly feasible in practice and it would almost certainly fail to cover a high number of individuals. In order to determine whether vaccination is possible or not in an Apennine context, we conducted this study with the aim of evaluating the uptake of ORV baits by target and non-target species in Central Italy. Our study aims to demonstrate that ORV campaigns cannot be planned without carefully analyzing beforehand the environmental, climatic and faunal factors present in the location where the campaign will be performed.

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